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**Citation for published version:**

Velders, FP, Kuningas, M, Kumari, M, Dekker, MJ, Uitterlinden, AG, Kirschbaum, C, Hek, K, Hofman, A, Verhulst, FC, Kivimaki, M, Van Duijn, CM, Walker, BR & Tiemeier, H 2011, 'Genetics of cortisol secretion and depressive symptoms: A candidate gene and genome wide association approach', *Psychoneuroendocrinology*, vol. 36, no. 7, pp. 1053-1061. <https://doi.org/10.1016/j.psyneuen.2011.01.003>

**Digital Object Identifier (DOI):**

[10.1016/j.psyneuen.2011.01.003](https://doi.org/10.1016/j.psyneuen.2011.01.003)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher's PDF, also known as Version of record

**Published In:**

*Psychoneuroendocrinology*

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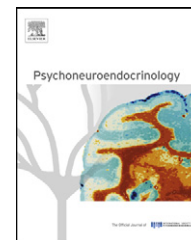




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# Genetics of cortisol secretion and depressive symptoms: A candidate gene and genome wide association approach

Fleur P. Velders<sup>a</sup>, Maris Kuningas<sup>d,g</sup>, Meena Kumari<sup>b</sup>,  
Marieke J. Dekker<sup>f</sup>, Andre G. Uitterlinden<sup>d,f,g</sup>, Clemens Kirschbaum<sup>c</sup>,  
Karin Hek<sup>d,h</sup>, Albert Hofman<sup>d,g</sup>, Frank C. Verhulst<sup>a</sup>, Mika Kivimäki<sup>b</sup>,  
Cornelia M. Van Duijn<sup>d,g</sup>, Brian R. Walker<sup>e</sup>, Henning Tiemeier<sup>a,d,g,\*</sup>

<sup>a</sup> Department of Child and Adolescent Psychiatry, Erasmus Medical Centre – Sophia Children's Hospital, Rotterdam, The Netherlands

<sup>b</sup> Department of Epidemiology and Public Health, University College London, United Kingdom

<sup>c</sup> Department of Psychology, Technical University of Dresden, Germany

<sup>d</sup> Department of Epidemiology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

<sup>e</sup> Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom

<sup>f</sup> Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands

<sup>g</sup> Netherlands Genomics Initiative (NGI) sponsored Netherlands Consortium for Healthy Aging (NCHA), The Netherlands

<sup>h</sup> Research Centre, Department of Psychiatry, Erasmus Medical Centre, Rotterdam, The Netherlands

Received 13 August 2010; received in revised form 8 December 2010; accepted 7 January 2011

## KEYWORDS

Salivary cortisol;  
Candidate gene;  
GWAS;  
FKBP5;  
Depressive symptoms

## Summary

**Background:** Depressive patients often have altered cortisol secretion, but few studies have investigated genetic variants in relation to both cortisol secretion and depression. To identify genes related to both these conditions, we: (1) tested the association of single nucleotide polymorphisms (SNPs) in hypothalamic–pituitary–adrenal-axis (HPA-axis) candidate genes with a summary measure of total cortisol secretion during the day (cortisol<sub>AUC</sub>), (2) performed a genome wide association study (GWAS) of cortisol<sub>AUC</sub>, and (3) tested the association of identified cortisol-related SNPs with depressive symptoms.

**Methods:** We analyzed data on candidate SNPs for the HPA-axis, genome-wide scans, cortisol secretion ( $n = 1711$ ) and depressive symptoms (the Centre for Epidemiology Studies Depression Scale, CES-D) ( $n = 2928$ ) in elderly persons of the Rotterdam Study. We used data from the Whitehall II study ( $n = 2836$ ) to replicate the GWAS findings.

**Results:** Of the 1456 SNPs in 33 candidate genes, minor alleles of 4 SNPs (rs9470080, rs9394309, rs7748266 and rs1360780) in the *FKBP5* gene were associated with a decreased cortisol<sub>AUC</sub>.

\* Corresponding author at: Department of Epidemiology, Erasmus Medical Centre, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. Tel.: +31 10 703 2183; fax: +31 10 704 4465.

E-mail address: [h.tiemeier@erasmusmc.nl](mailto:h.tiemeier@erasmusmc.nl) (H. Tiemeier).

( $p < 1 \times 10^{-4}$ ) after correction for multiple testing using permutations). These SNPs were also associated with an increased risk of depressive symptoms (rs9470080: OR 1.19 (95%CI 1.0; 1.4)). The GWAS for cortisol yielded 2 SNPs with  $p$ -values of  $1 \times 10^{-6}$  (rs8062512, rs2252459), but these associations could not be replicated.

**Conclusions:** These results suggest that variation in the *FKBP5* gene is associated with both cortisol<sub>AUC</sub> and the likelihood of depressive symptoms.

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## 1. Introduction

The diurnal secretion of cortisol is characterized by high levels in the morning followed by a decline in cortisol levels toward the evening, and reflects the total cortisol exposure during day time. After its release from the adrenal glands, cortisol exerts diverse actions including on the immune system, glucose metabolism and the central nervous system. The effect of cortisol on target organs is mediated by two types of receptor: the mineralocorticoid receptor (MR (NR3C12)) and the glucocorticoid receptor (GR (NR3C1)). In the brain, MR mediates the onset of the stress response, whereas GR is involved in the termination of the stress response. The heritability of diurnal cortisol secretion has been estimated at 62% (Bartels et al., 2003).

Several studies have shown that depression is associated with hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis resulting in high cortisol levels (Holsboer, 2000; Ehlert et al., 2001; Vreeburg et al., 2009) and abnormal cortisol secretion in response to standard stimuli, such as the DEX/CRH suppression test (Heuser et al., 1994). It has been suggested that these alterations in cortisol secretion are causally related to the development of depression and for this reason the identification of genes related to cortisol secretion may enhance the discovery of genes associated with depression (Gottesman and Gould, 2003). Recent studies provide some support for this hypothesis. For example, single nucleotide polymorphisms (SNPs) in the MR gene have been associated with morning cortisol levels (van Leeuwen et al., 2009) and, in elderly subjects, with the prevalence of depressive symptoms (Kuningas et al., 2007). SNPs within the GR gene have been associated with hypersensitivity to cortisol, the occurrence of depression, and the response to antidepressant treatment (Spijker and van Rossum, 2009). The FK506 binding protein 5 (FKBP5), which acts as co-chaperone of GR, is associated with the sensitivity of GR to cortisol and SNPs within the *FKBP5* gene have been shown to be associated with depression (Lekman et al., 2008; Zobel et al., 2010), treatment response and recurrence of depressive episodes (Binder et al., 2004). However, most of these studies examined cortisol secretion under stressful circumstances without an assessment of basal cortisol secretion. In the current study, we sought to find genes related to cortisol secretion and ultimately to depressive symptoms. We measured saliva cortisol concentrations during the day, which approximate free plasma cortisol (Kirschbaum and Hellhammer, 1994; Gozansky et al., 2005). To prevent multiple testing and to increase precision, we applied a commonly used summary measure of total cortisol secretion during the day expressed as the area under the curve (cortisol<sub>AUC</sub>) (Pruessner et al., 2003). First, we performed a candidate gene study of cortisol<sub>AUC</sub>. Candidate gene studies rely on biological knowledge

and are able to examine the postulated association with more power as multiple testing is not so stringent as less tests are performed due to prior selection. However, this approach does not identify new genes. Therefore, we also performed a hypothesis free GWAS to try to discover genes not previously associated with HPA-axis functioning. Third, we examined the effect of the SNPs associated with cortisol<sub>AUC</sub> in the candidate gene study and/or GWAS on the risk of depressive symptoms.

## 2. Methods and materials

### 2.1. Setting and study population

This study is embedded in the Rotterdam Study, an ongoing population-based cohort on risk factors for chronic diseases in the elderly. Detailed information on design, objectives and methods has been presented elsewhere (Hofman et al., 2009). The Medical Ethics Committee of the Erasmus Medical Center approved the Rotterdam study and written informed consent was obtained from all participants. Of the 3550 participants of the fourth study survey (2002–2004), genetic and cortisol data were available in 1711 persons. In 2928 participants, genetic data and information about depressive symptoms were available.

### 2.2. Candidate genes and genome-wide data

Based on the literature, we selected candidate genes known to be involved in central regulation of the HPA-axis, cortisol biosynthesis in the adrenal, or the clearance of cortisol from the circulation (Table 2). To select SNPs within the candidate gene regions, we included 100 kb at 5' and 3' of the genes to e.g. include regulatory regions. We then extracted these SNPs from the GWAS genotyping, using PLINK v1.02 (Purcell et al., 2007).

The genome-wide genotyping was done with the Illumina 550K array (Illumina, San Diego, CA, USA) in self-reported Caucasian individuals (sample call rate  $\geq 97.5\%$ ) (Richards et al., 2008). We excluded individuals for excess autosomal heterozygosity, mismatch between genotypic and phenotypic gender, and outliers identified by the identity-by-state (IBS) clustering analysis. SNPs were excluded when the minor allele frequency (MAF) was 1% or less, the Hardy–Weinberg equilibrium (HWE)  $p$ -value was smaller than  $1 \times 10^{-5}$ , or the SNP call rate was 90% or less. This resulted in 530,683 SNPs.

### 2.3. Cortisol collection

Participants were asked to collect saliva samples at home using Salivette sampling devices (Sarstedt, Rommelsdorf,

Germany). As described previously (Dekker et al., 2008), participants received detailed oral and written instructions concerning the saliva sampling, and were asked to collect four saliva samples during one single weekday: at awakening, 30 min later, at 5 pm and at bedtime. Samples were stored in the freezer at  $-80^{\circ}\text{C}$  and later sent to the laboratory of Biopsychology, TU Dresden, Germany. Salivary cortisol concentrations were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL Hamburg, Germany). Intra- and interassay coefficients of variation were below 6% and 9%, respectively. In line with a previous study (Dekker et al., 2008), cortisol values above the 98th percentile in the original cortisol dataset were excluded.

## 2.4. Depressive symptoms

Depressive symptoms were assessed using the Centre for Epidemiology Studies Depression Scale (CES-D) which is a validated and reliable self-report measure (Radloff, 1977). The CES-D consists of 20 items with total scores ranging from 0 to 60. We used a cut-off of 16 or higher to dichotomize depressive symptoms. This cut-off is indicative of clinically relevant symptoms and a sensitive threshold for depressive disorder (Beekman et al., 1997).

## 2.5. Other covariates

Information about gender, age, educational level, smoking habits was assessed by interview, coronary heart disease (fatal or nonfatal myocardial infarction, a percutaneous transluminal coronary angioplasty, a coronary artery bypass graft, other forms of acute or chronic ischemic heart disease, sudden cardiac death, and death due to ventricular fibrillation and congestive heart failure), diabetes mellitus type II was assessed by fasting glucose levels and the use of anti-diabetic medication, use of hypnotics, antidepressants and antipsychotics was assessed by cabinet check.

## 2.6. GWAS replication study

The Whitehall II Cohort recruited participants between 1985 and 1988 (phase 1) from 20 London-based civil service departments. Data reported here are from the phase 7 (2002–2004) data collection when large-scale genotyping was undertaken for the first time. Saliva samples were returned by 90.1% ( $n = 4609$ ) of the participants who attended the screening clinic (Badrick et al., 2007). Details of the clinical assessment and cohort profile have been reported elsewhere (Marmot and Brunner, 2005).

The protocol of saliva collection has been described previously (Badrick et al., 2007). Briefly, participants were requested to provide six saliva samples over the course of a normal weekday at awakening, waking +30 min, waking +2.5 h, waking +8 h, waking +12 h and bedtime. Salivary cortisol levels were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL Hamburg, Germany).

SNPs were genotyped at kbioscience (<http://www.kbioscience.co.uk>) using KASPar chemistry, which is a competitive allele-specific PCR SNP genotyping system using FRET quencher cassette oligos. Blind duplicates, plate-identifying

blank wells and Hardy–Weinberg equilibrium tests were used as quality control tests. Cortisol data and genotype data were available in 2836 white participants of the Whitehall II Study.

## 2.7. Statistical analyses

First, we determined the total diurnal cortisol secretion with respect to the ground expressed by the area under the curve (cortisol<sub>AUC</sub>). This is given by the cortisol measurements in nmol/L on the y-axis and the time between the cortisol measurements on the x-axis (Pruessner et al., 2003). To correct for differences in length of total sampling interval, the cortisol<sub>AUC</sub> was divided by time awake during the day in hours. The unit of cortisol<sub>AUC</sub> is thus (nmol  $\times$  h/L)/h = nmol/L.

Second, we performed a linear regression analysis to examine the association of SNPs of candidate genes with the cortisol<sub>AUC</sub>. *p*-values of these associations were corrected for multiple testing using the Max(T) permutation procedure (10,000 permutations). The regression analysis and the permutation procedure were performed using PLINK v1.02 (Purcell et al., 2007).

Third, we performed a genome-wide quantitative trait analysis of diurnal cortisol secretion using PLINK v1.02 (Purcell et al., 2007). The Hardy–Weinberg equilibrium (HWE) *p*-value was set at  $1 \times 10^{-4}$ , the maximum percentage SNPs per person missing at 2%, and the threshold for the minor allele frequency (MAF) at 1%. We also performed a GWAS in females and males separately.

Fourth, to replicate our GWAS findings we selected SNPs of the GWAS based on *p*-values ( $< 1 \times 10^{-5}$ ) and LD between SNPs. We also selected SNPs of the GWAS that were located in candidate genes and showed a strong association (*p*-value  $< 1 \times 10^{-4}$ ) with the cortisol<sub>AUC</sub>. We tested the association of these selected SNPs of the GWAS with cortisol data of the Whitehall II Study using linear regression analyses in an additive model (SPSS Inc., Chicago, IL, USA). Next, we performed a meta-analysis using the inverse Z score method assuming fixed effects (R 2.8.1).

Fifth, we tested the association of statistically significantly associated SNPs in the candidate gene study or the GWAS with clinically relevant depressive symptoms in participants of the Rotterdam Study using logistic regression analysis in PLINK v1.02 (Purcell et al., 2007), adjusted for age and gender. To examine if the association of SNPs and depressive symptoms could be explained by other characteristics, we tested if non-carriers and carriers differed with respect to gender, age, educational level, smoking, diabetes mellitus type II, coronary heart disease, or the use of hypnotics, antidepressants and antipsychotics.

## 3. Results

### 3.1. GWAS sample and replication sample

Table 1 presents the characteristics of participants of the GWAS discovery sample and the replication sample. Participants of the GWAS discovery sample were more likely female, and were, on average, older than participants in the replication sample. Furthermore, people in the GWAS discovery sample were more likely to smoke, less highly educated, and were more likely to report diabetes mellitus type II,



**Table 1** Characteristics of Rotterdam Study participants and Whitehall II Study participants with cortisol data.

	Rotterdam study (n = 1711) <sup>a</sup>	Whitehall II study (n = 2836) <sup>a</sup>
Gender (male)	45.4%	77.7%
Age (yrs.)	74.9(5.7) <sup>b</sup>	60.9 (5.9) <sup>b</sup>
Education		
University/polytechnic	12.0%	37.1%
Smoking	12.5%	7.7%
Diabetes mellitus Type II	5.4%	6.6%
Coronary heart disease	13.2%	4.5%
Hypnotics	14.1%	1.2%
Antidepressants/antipsychotics	4.5%	4.0%
Cortisol (nmol/L)		
At awakening	13.17(0.01–42.97)	14.80(0.10–40.05)
30 min. after awakening	16.80(0.06–51.12)	21.82(0.01–54.52)
8 h. after awakening	3.42(0.01–19.09)	5.13(0.00–19.50)
At bedtime	1.64(0.02–13.42)	1.50(0.00–13.59)
Cortisol <sub>AUC</sub> (nmol/L)	8.14(3.47) <sup>b</sup>	6.93(2.29) <sup>b</sup>

Numbers are median (100% range) unless otherwise indicated.

<sup>a</sup> *t* test and Mann–Whitney test for continuous variables and Chi-square tests for dichotomous variables.

<sup>b</sup> Mean (standard deviation).

coronary heart disease and the use of hypnotics. Also, there were small differences in cortisol levels between the two samples.

### 3.2. The candidate gene study

The list of 33 candidate genes, the number of SNPs within these genes (1456 in total), and their position are presented in Table 2. Initially, 96 of 1456 SNPs were associated with the cortisol<sub>AUC</sub> with a *p*-value less than or equal to 0.05 (see supplementary material (SM) Table 2), of which Table 3 shows the top 10 SNPs located in 7 genes; the FK506 binding protein 5 (*FKBP5*) gene, the melanocortin 4 receptor (*MC4R*) gene, the corticotrophin-releasing hormone receptor 2 (*CRHR2*) gene, the arginine vasopressin (*AVP*) gene, the steroid-5- $\alpha$ -reductase (*SRD5A1*) gene, the mineralocorticoid receptor (*NR3C2*) gene, and the 3- $\beta$  hydroxysteroid dehydrogenase II (*HSD3B2*) gene. After adjustment for multiple testing, 4 SNPs (rs9470080, rs9394309, rs7748266 and rs1360780) showed significantly lower cortisol<sub>AUC</sub> per minor allele. These SNPs are all located in the *FKBP5* gene and in strong LD with each other.

### 3.3. GWAS of cortisol and the replication study

Table 4 presents the results of the GWAS, the replication and the meta-analysis. The most significant association was found between rs8026512 on chromosome 15 with a lower cortisol<sub>AUC</sub> per C allele ( $\beta = -0.57$ , *p*-value  $6.04 \times 10^{-6}$ ). The second hit (rs11630255) was in LD with rs8026512 and showed a similar association with cortisol ( $\beta = -0.57$ , *p*-value  $7.45 \times 10^{-6}$ ) so was not selected for replication. The third hit with a *p*-value  $< 1 \times 10^{-5}$  (rs2252459) was associated with a higher cortisol<sub>AUC</sub> per C allele ( $\beta = 0.53$ , *p*-value  $8.75 \times 10^{-6}$ ). It is located in intron 3 of the activated leukocyte cell adhesion molecule (*ALCAM*) gene on chromosome 3 (3q13.1). There were no other hits with a *p*-

value  $< 1 \times 10^{-5}$ . Within the first ten hits of the GWAS, we found two SNPs (rs9470080 and rs9394309) that were also identified in the candidate gene study above. These SNPs, located in the *FKBP5* gene region, were associated with a lower cortisol<sub>AUC</sub> per risk allele (rs9470080:  $\beta = -0.55$ , *p*-value  $1.26 \times 10^{-5}$ ; rs9394309:  $\beta = -0.56$ , *p*-value  $1.58 \times 10^{-5}$ ). The first 50 hits of the GWAS, the Q-Q plot and Manhattan plot are included in the SM (see (SM) Table 3 and Figs. 1 and 2). The GWAS stratified on gender (776 males and 931 females) yielded less SNPs with *p*-values of  $1 \times 10^{-5}$  or smaller than the GWAS in the whole sample. Two top SNPs of the GWAS in the whole sample were also found among the top hits in the stratified analyses; males rs9470080 *p*-value  $9.95 \times 10^{-5}$ , females rs8026512 *p*-value  $1.94 \times 10^{-5}$ . The results of the stratified analyses are presented in Table 4a (males) and Table 4b (females) of the supplementary material.

To replicate our main findings, top hit SNPs (rs8026512, rs2252459, rs9470080 and rs9394309) were genotyped in the Whitehall II study. Of the four SNPs, two (rs8026512 and rs2252459) were associated with the cortisol<sub>AUC</sub> in the replication cohort; however, the effects were in the opposite direction. Hence, none of the initial SNPs associated with cortisol secretion in the GWAS sample were successfully replicated.

The association of candidate genes and clinically relevant depressive symptoms.

Table 5 presents the association between the 4 SNPs which remained significant after adjustment for multiple testing in the candidate gene analysis, and depressive symptoms. Since these SNPs are in strong LD, our results were not corrected for multiple testing. Carriers of minor alleles of rs9470080 were at an increased risk of depressive symptoms (OR 1.19, 95%CI 1.01; 1.40, *p*-value 0.037).

The distribution of gender, age, educational level, smoking, DM type II, CHD and use of hypnotics did not differ between carriers of rs9470080 and non-carriers. In contrast, carriers of

**Table 2** Selected candidate genes expected to explain variation in cortisol secretion.

Gene Symbol <sup>a</sup>	No. of SNPs (Illumina550k)	Position
<b>Corticosteroid biosynthesis</b>		
CYP21A2	35	6:32014061-32217398
CYP11B1	35	8:143850775-144058238
CYP17A1	35	10:104480278-104687280
HSD3B2	39	1:119659296-119867174
CYP11A1	48	15:72317156-72547134
MC2R	48	18:13774624-13975517
STAR	7	8:38019375-38227757
PRKAR1A	41	17:63923130-64159026
<b>Central HPA-axis control &amp; negative feedback</b>		
NR3C1	58	5:142537689-142863447
NR3C2	104	4:149120957-149677462
AVP	45	20:2911203-3113370
AVPR1A	44	12:61726483-61932857
CRH	24	8:67,251,173- 67,253,252
CRHR1	47	17:41117449-41368973
CRHR2	53	7:30559388-30788665
POMC	34	2:25137226-25345063
MC4R	48	18:56089544-56290981
POU1F1	42	3:87291473-87508427
PROP1	26	5:177251842-177455849
TBX19	49	1:166416902-166650288
LEPR	82	1:65558906-65975410
LEP	37	7:127568567-127784918
FKBP5	48	6:35549346-35864692
<b>Cortisol inactivation and metabolism</b>		
HSD11B2	8	16:65922537-66128955
HSD11B1	58	1:207826173-208074918
DHRS9	32	2:169534209-169746650
SRD5A1	78	5:6586500-6822675
SRD5A2	20	2:31503160-31759544
AKR1D1	56	7:137327054-137553590
CYP3A4	20	7:99092540-99319744
H6PD	55	1:9117450-9353981
SERPINA6	73	14:93740338-93959441
ACE	26	17:58808166-59048711
33 genes	1456 SNPs	

Abbreviations: SNP, single nucleotide polymorphism.

<sup>a</sup> Full gene names are given in [supplementary material \(SM\) Table 1](#).

rs9470080 were more frequently using antidepressants or antipsychotics compared with non-carriers (SM Table 5).

#### 4. Discussion

This study from two well-characterized European cohorts of middle-aged or elderly adults used candidate gene and genome wide approaches to identify genes associated with diurnal cortisol secretion and their association with depressive symptoms. We found evidence for an association of *FKBP5* SNP rs9470080 with both saliva cortisol concentrations and depressive symptoms.

FKBP5 is a co-chaperone of hsp90, which is part of a receptor complex that regulates the sensitivity of the glu-

cocorticoid receptor (GR). An increase in FKBP5 gene expression leads to an increased resistance of GR to cortisol, which may result in hypercortisolism (Binder, 2009). GWAS and linkage studies, one of which includes data from the Rotterdam Study, did not report SNPs within the FKBP5 gene as one of the most significant associations (Bosker et al., 2010; Muglia et al., 2010; Schol-Gelok et al., 2010). In previous candidate gene studies, *FKBP5* SNPs have been associated with depression (Lekman et al., 2008; Zobel et al., 2010), the recurrence of depressive episodes, the response to antidepressant treatment (Binder et al., 2004; Zou et al., 2010), peritraumatic dissociation in children (Koenen et al., 2005), bipolar disorder (Willour et al., 2009), and suicide behaviour (Brent et al., 2010; Roy et al., 2010). FKBP5 gene expression was associated with posttraumatic stress disorder (PTSD) (Yehuda et al., 2009). Furthermore, carriers of FKBP5 SNPs showed insufficient recovery of cortisol levels after psychosocial stress (Ising et al., 2008). Also, interaction effects have been found with FKBP5 SNPs and childhood abuse on PTSD (Binder et al., 2008) and FKBP5 SNPs and resistant attachment on cortisol reactivity in infants (Luijk et al., 2010). Most studies examined the effect of *FKBP5* SNPs under stressful circumstances only. Such a paradigm may activate different regulatory mechanisms than those regulating basal cortisol secretions.

In the present study, carriers of genetic variants in the *FKBP5* gene had a lower cortisol<sub>AUC</sub> and an increased risk of depressive symptoms compared with non-carriers. The latter finding is in line with the observation that TT-carriers of the *FKBP5* rs1360780 variant are more likely to have recurrent and treatment-refractory depression. However, depression is typically associated with high cortisol levels. Several aspects, other than a chance finding, must be considered when interpreting these findings. Firstly, the relation between depression and hypercortisolism is less consistent than frequently stated. In a meta-analysis, Burke and colleagues showed that the association between depression and cortisol levels at baseline strongly depends on the time of day. In the morning, depressed patients show lower cortisol levels than non-depressed individuals. In the afternoon, cortisol levels are higher among depressed patients compared to non-depressed individuals. Also, Oldehinkel et al. (2001) found that hypocortisolism, and not hypercortisolism, was related to chronic depressive episodes. Furthermore, Penninx et al. (2007) reported a U-shaped relation between depression and cortisol among elderly subjects. In this study, depressed elders with the lowest cortisol levels also scored higher on frailty indicators than depressed elders with cortisol levels in the highest tertile. Furthermore, hypocortisolism has been associated with depression in elderly females (Bremner et al., 2007). This may explain the association of FKBP5 SNPs with lower cortisol<sub>AUC</sub> and depression in our study, since our study of elderly was predominantly female. Second, depression in community-dwelling elderly is typically more chronic as a consequence of the ascertainment, but acute and clinical depression is characterized by high levels of cortisol. Third, we studied depressive symptoms and not a clinical diagnosis of depression or subtypes of depression. However, the observation that carriers of FKBP5 rs9470080 were more likely to report clinically relevant depressive symptoms and also more likely to use antidepressants or antipsychotics further indicates that depressive symptoms are a good marker of severe

**Table 3** The association of candidate gene SNPs with cortisol<sub>AUC</sub>.

SNP		Gene	Chr.	Variant	MAF	beta	se	p-value unadj.	p-value adj. <sup>a</sup>
Most significant	No. significant								
Cortisol <sub>auc</sub> (nmol/L)									
rs9470080	4	FKBP5	6	G > A	0.34	−0.55	0.12	1.0e−05	0.001
rs11152221	—	MC4R	18	G > A	0.32	−0.36	0.12	3.1e−03	0.18
rs2284218	—	CRHR2	7	A > G	0.36	0.34	0.12	5.6e−03	0.35
rs2296236	—	AVP	20	G > A	0.16	−0.44	0.16	7.1e−03	0.40
rs7735244	—	SRD5A1	5	A > G	0.10	−0.50	0.19	9.6e−03	0.44
rs10519942	—	NR3C2	4	G > A	0.07	0.59	0.23	0.009	0.48
rs6672903	—	HSD3B2	1	A > G	0.44	0.30	0.12	0.012	0.59

Abbreviations: SNP, single nucleotide polymorphism; Chr., chromosome; MAF, minor allele frequency; se, standard error.

<sup>a</sup> Adjusted for multiple testing using Max(T) permutation (10,000).

**Table 4** Main findings GWAS cortisol<sub>AUC</sub> and replication results.

SNP	Gene	Chr.	Variant	Rotterdam Study (n = 1711)		Whitehall II Study (n = 2836)		Meta-analysis (n = 4547)	
				beta	p-value	beta	p-value	beta	p-value
Cortisol <sub>AUC</sub> (nmol/L)									
rs8026512	—	15	T > C	−0.57	6.04e−06	0.13	0.037	−0.01	0.830
rs2252459	ALCAM	3	T > C	0.53	8.75e−06	−0.13	0.039	0.01	0.803
rs9470080	FKBP5	6	G > A	−0.55	1.26e−05	−0.025	0.702	−0.14	0.017
rs9394309	FKBP5	6	A > G	−0.56	1.58e−05	−0.006	0.927	−0.12	0.037

Abbreviations: SNP, single nucleotide polymorphism; Chr., chromosome.

psychiatric disorders. Also, we did not rule out persons with depressive symptoms due to PTSD, fibromyalgia or chronic fatigue, which have been associated with hypocortisolism (Fries et al., 2005). Finally, the relation between *FKBP5* variants and depression could not be explained by the cortisol parameters in the present study as there was no association between cortisol<sub>AUC</sub> and depressive symptoms. This can reflect a lack of power, but could also indicate that another mechanism than basal cortisol regulation underlies the observed associations.

The GWAS approach identified two SNPs which are potentially relevant for cortisol secretion. Our top hit (rs8026512) was located on chromosome 15 in the Prader Willi syndrome (PWS) deletion region. This syndrome is a neurobehavioural disorder characterized by hypertonia, failure to thrive,

hyperphagia, short stature, hypogonadism and developmental delay. de Lind van Wijngaarden et al. (2008) reported a 60% prevalence of central adrenal insufficiency in PWS patients, which is characterized by insufficient cortisol. The second hit (rs2252459) is located in intron 3 of the activated leukocyte cell adhesion molecule (ALCAM) gene on chromosome 3 (3q13.1). This cell adhesion molecule is expressed by epithelial cells in several organs. Alterations in the expression of ALCAM have been reported in several human tumours (Ofori-Acquah and King, 2008). Although it is not directly clear how ALCAM may influence cortisol secretion, there may be a link between ALCAM and cortisol via the immune system.

The replication of the association of four SNPs with cortisol<sub>AUC</sub> from the GWAS in the Rotterdam Study (RS) was not

**Table 5** Results of the association of *FKBP5* SNPs with depressive symptoms (n = 2928).

Chr.	SNP	Position	Variant	CES-D			LD with rs9470080	
				Depressive symptoms score ≥ 16 <sup>a</sup> n = 375			ΔR <sup>2</sup>	D'
				OR	95%CI	p-value		
6	rs9470080	35754413	G → A	1.19	1.01;1.40	0.037	—	—
6	rs9394309	35729759	A → G	1.15	0.97;1.35	0.107	0.95	1
6	rs7748266	35700722	G → A	1.16	0.95;1.43	0.151	0.46	1
6	rs1360780	35715549	G → A	1.18	1.00;1.39	0.057	0.84	1

Abbreviations: CES-D, Centrum for Epidemiology Studies Depression Scale; LD, linkage disequilibrium; Chr, chromosome; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

<sup>a</sup> Reference = CES-D depressive symptoms score < 16.

successful in the Whitehall II study. The associations found in the replication sample were not in the same direction and had thus not been hypothesized based on the initial GWAS. They were also not significant after Bonferroni correction.

Inconsistent findings may be the result of false-positive findings in the GWAS, or falsely non-replicated true GWAS findings in the replication sample (Ioannidis, 2007). Due to the number of tests performed in a GWAS, the frequency of false-positive findings is high. In this study with 1711 participants, we had sufficient power (0.80) to detect an  $R^2$  change of 0.0045 (Quanto 1.2.4 (Gauderman, 2003,2006)). We had 0.55 power to detect the effect sizes (beta =0.56) associated with our strongest hits. Furthermore, phenotyping and genotyping errors may account for false-positive findings in a GWAS. However, in both studies cortisol data were obtained by saliva sampling at home with several samples on one day, and cortisol levels were assessed in the same laboratory. Albeit statistically significant, the differences in cortisol levels were small. Hence, the phenotype definition is unlikely to account for the non-replication. The other differences between the two studies are likely the results of the difference in inclusion criteria of both studies. The WHII Study consists of civil servants from London which implies that the study population has, on average, a high educational level and more men. This high social economic status may account for differences in the prevalence of disease, and the use of hypnotics. It has been reported that cortisol levels increase with age (Van Cauter et al., 1996; Seeman et al., 2001). Also, there is evidence to suggest that cortisol levels are higher in older men than older women (Seeman et al., 2001). Grant et al. (2007) reported higher urinary free cortisol levels in depressed men than in depressed women. These urinary free cortisol levels in depressed males further increased with age and severity of the illness. As the participants of the Rotterdam Study and the Whitehall II Study were all older adults, it seems unlikely that difference in age accounts for the non-replication. Gender distribution in the two cohorts was different with a majority of female participants in the Rotterdam Study and a majority of males in the replication sample. However, the GWAS of cortisol<sub>AUC</sub> stratified on gender did not yield associations with lower *p*-values, which is likely due to insufficient power. The GWAS and the replication study were not adjusted for other covariates related to cortisol levels, since SNP associations are very unlikely to be confounded by these variables. This is because most confounding factors do not alter an individual's genetic make-up. Adjusting for covariates that affect cortisol secretion may increase precision of our phenotype, but could also blunt the measured gene-phenotype association (Koepsell and Weiss, 2003).

Next to cortisol<sub>AUC</sub>, the single cortisol measurements and the cortisol awakening response (CAR) are frequently used phenotypes of cortisol secretion. In this study, we focused on the cortisol<sub>AUC</sub>, because this daytime profile gives more information about the HPA-axis activity than the single cortisol measurements and reduces the risk of multiple testing (Pruessner et al., 2003). The CAR was not used as outcome measurement, because its exact function remains to be determined and conflicting results, both an elevated CAR as well as a blunted CAR, in relation to depression have been found (Fries et al., 2009).

Next to false-positive findings, it could be that our GWAS findings were falsely non-replicated in the WHII study. This

may be due to phenotyping and genotyping errors or low power. As discussed previously, the phenotyping in the two samples was very comparable. Also, all SNPs were in HWE and there was no miscoding of major versus minor alleles. To improve power, replication studies with substantially larger sample sizes are needed to reliably determine whether we observed true associations or chance findings in the GWAS of cortisol<sub>AUC</sub>.

At least two other studies have used a genome wide approach to identify genes associated with cortisol secretion. Ukkola et al. (2002) performed a genome wide linkage scan on morning serum cortisol in black and white families, but did not find evidence for linkage between any of the microsatellite markers and cortisol. A subsequent family-based linkage study identified a significant association between morning serum cortisol and two markers on chromosome 11 and 14 in women (Kurina et al., 2005). Although saliva cortisol levels and serum cortisol levels are highly correlated (Kirschbaum and Hellhammer, 1994; Gozansky et al., 2005), morning levels of cortisol tend to be highly variable due to the steep morning rise and do not represent cortisol<sub>AUC</sub> well. Therefore, the lack of power to identify complex gene variants using the linkage approach, and the difference in phenotype definition are likely to account for the lack of congruence between these two studies and our study.

In conclusion, we found an association of *FKBP5* SNPs with a decrease in cortisol<sub>AUC</sub> in a population-based cohort. Carriers of *FKBP5* minor alleles were also at increased risk of clinically relevant depressive symptoms. Although this is consistent evidence for the physiological and clinical relevance of variants in this HPA-axis regulating gene in an epidemiological study, future laboratory studies are needed to establish the causal mechanism behind these associations.

## Role of funding source

The generation and management of the genotype data for the Rotterdam Study are supported by the Netherlands Organization of Scientific Research Investments (#175.010.2005.011, 911-03-012). The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam; Netherlands Organization for the Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE and RIDE2); the Ministry of Education, Culture and Science; the Ministry for Health, Welfare and Sports; the European Commission (DG XII); the Municipality of Rotterdam; and the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research project number 050-060-810. F.P. Velders is supported by the Sophia Foundation for Scientific Research (SKZ Foundation) (grant no. 491) and Netherlands Organization for the Health Research and Development (ZonMw) (grant no. 10.000.1003).

The WH-II study has been supported by grants from the Medical Research Council; British Heart Foundation; Health and Safety Executive; Department of Health; National Institute on Aging, NIH, US (AG13196); Agency for Health Care Policy Research (HS06516); and the John D. and Catherine T. MacArthur Foundation Research Networks on Successful Mid-life Development and Socio-economic Status and Health. M. Kumari is partially supported by the National Heart Lung and Blood Institute (NHLBI: HL36310).



Prof. B.R. Walker is supported by the British Heart Foundation and the Chief Scientist Office of the Scottish Government.

These funding sources had no further role in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

## Conflict of interest

All authors declare that they have no conflict of interest.

## Acknowledgements

We thank Pascal Arp, Mila Jhamai, Dr. Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the genome-wide association study database. We are grateful to the study participants, the staff from the Rotterdam Study, and the participating general practitioners and pharmacists.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.psyneuen.2011.01.003](https://doi.org/10.1016/j.psyneuen.2011.01.003).

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